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Rapid Trichrome Stain

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A rapid trichrome stain procedure was evaluated. It saves considerable time, especially when only a few slides are stained, and allows for the accurate identification of amoebae and other fecal parasites.

The examination of permanently stained fecal preparations is recommended for the accurate identification of intestinal parasites (1). Stained slides provide direct evidence of parasites and cellular exudates, allow for consultation with other microscopists, and can be used for educational purposes. The Wheatley trichrome method (3) is one of the most popular and common procedues in parasitology. However, two disadvantages of the conventional trichrome stain (2) are: (i) it takes at least 42 min to perform and (ii) reagents in Koplin jars can become contaminated and weakened during repeated usage. The minimum staining time of 42 min is probably the most critical disadvantage, especially in microbiology laboratories with limited personnel. If the procedure was more rapid, laboratorians could save time and money and would therefore tend to use permanent stains more often. The purpose of this study, therefore, was to evaluate a new rapid trichrome stain procedure.

Reagents for the procedure were identical to those used in the conventional method (2); however, duplicate solutions of 70 and 95% alcohol were replaced with single solutions. All of the solutions were placed in 250-ml plastic squeeze bottles. Reagents were added to slides, dropwise over a sink, as is often done with the Gram stain. A slide warmer is required and is used to rapidly volatilize the solutions and dry the slide material between several steps.

The rapid trichrome stain procedure is as follows. (i) Prepare a thin fecal smear with polyvinyl alcohol and let it air dry overnight. (ii) 70% alcohol-iodine: add about 20 drops, with slide tilted down (over sink), until material to be stained is fully saturated. Dry on a slide warmer at 60 to 65°C until all observable moisture is gone. (iii) 70% alcohol: add about 10 drops to a tilted slide and dry on a slide warmer. (iv) trichrome stain: saturate the material to be stained and let the slide set level for at least 2 to 3 min. Do not dry on a slide warmer. After staining, tilt the slide to allow the excess stain to run off. (v) 90% acidified alcohol: tilt the slide down and add about 5 drops of alcohol, saturating the material to be stained. Wipe off the excess liquid at the bottom of the tilted slide and dry the slide on the slide warmer. (vi) 95% alcohol: tilt the slide and add about 5 to 10 drops of alcohol. Dry on the slide warmer. (vii) carbol-xylene: tilt a slide down and saturate material with about 5 to 10 drops of carbol-xylene solution. Do not dry on a slide warmer. (viii) xylene: add about 20 to 25 drops of xylene to a tilted slide until the carbol-xylene (oily appearance) runs off. While the slide material is still saturated with xylene, add the mounting fluid and a cover slip.

Fresh and stock fecal specimens, preserved in polyvinyl alcohol, were used to make duplicate smears. One smear was stained by the conventional method, and the other was stained by the rapid method. The slides were given a code number and subsequently evaluated by two experienced parasitologists. Results from the evaluations were then compared for accuracy.

The following parasites were observed microscopically during the trial period: *Entamoeba coli* cysts (C) and trophozoites (T), *Entamoeba histolytica* (C and T), *Entamoeba hartmanni* (C and T), *Endolimax nana* (C and T), *Chilomastix mesnili* (C and T), *Giardia lamblia* (C and T), *Dientamoeba fragilis* (T), and *Iodamoeba butschlii* (C and T).

Forty-four smears, all with parasites, were observed. When parasites were observed with conventional and rapid stains, there appeared to be no notable difference in clarity or number of parasites per microscopic objective field. In the rapid stain, peripheral chromatin and karyosomes stained a rose red; identification was similar, if not easier, than with the conventional stain. Also in the rapid stain, cytoplasm was differentiated from background material well, staining blue-green, with little distortion, in contrast to a green background. By the rapid method, one slide could be stained in less than 10

min. When a batch of 5 to 10 slides were stained, the rapid method was still quicker than the conventional method.

The rapid trichrome stain appears to be an effective and accurate method of staining preserved fecal smears. It requires considerably less time, when less than 10 slides are to be stained, and eliminates reagent contamination. When many specimens are to be stained, the conventional method may be faster because it does not require individual manipulation of slides. We wholeheartedly recommend that other investigators, using the conventional trichrome stain method, make comparisons with the rapid method. Our recommendations are based on comparatively few observations, and more experience is needed with *E. histolytica*, *E. hartmanni*, and *D. fragilis*. However, the rapid method appears to be very cost and time efficient and could therefore be very useful in laboratories that process only a few specimens daily.

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